PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

HEADQUARTERS
501 FRONT STREET
NORFOLK, VA 23510
TEL 757-622-PETA
FAX 757-622-0457

October 9, 2003

Marianne L. Horinko, Acting Administrator
U.S. Environmental Protection Agency
Ariel Rios Building (1101A)
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Re: Comments on the HPV test plan for 2,5-furandione, 3-(docenyl)dihydro-, reaction products with propylene oxide (2,5-fu)

Dear Acting Administrator Horinko:

The following are comments on the HPV test plan for 2,5-fu (CAS no. 68411-58-5), submitted by the American Chemistry Council (ACC). These comments are submitted on behalf of People for the Ethical Treatment of Animals, the Physicians Committee for Responsible Medicine, the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These animal, health and environmental protection organizations have a combined membership of more than ten million Americans.

The ACC reports the results of acute toxicity tests with this substance in which animals were dosed with what would be the equivalent of pumping more than 1.5 lbs of lubricating oil into a human's stomach. The LD-50 appears to be equivalent to or greater than the limit dose, i.e. Category IV non-toxic. Nevertheless, the ACC is planning to conduct an acute fish toxicity test (OECD 203), a combined repeat-dose, developmental and reproductive toxicity test (OECD 422), and an *in vivo* micronucleus genotoxicity test (OECD 474). These tests will kill at least 875 animals.

The ACC has apparently failed, once again, to take into account the fact that in the case of many lubricant additives, including the compounds in this category, the high molecular weight, low solubility, and the fact that they are diluted in a relatively non-toxic oil base in most exposure scenarios, limits the toxicity and bioavailability of these compounds and renders a more detailed toxicity analysis essentially moot. In the screening and prioritization process of the HPV program, it strikes us that the analysis of the toxicity of this category and similar compounds is quite easily identified without conducting further unreliable and cruel animal tests. Conducting additional tests for compounds such as these merely serves the check-the-box mentality which EPA purports to disavow.

Our specific comments are as follows:

First, the acute fish toxicity test is clearly inappropriate, because the octanol/water partition coefficient is too high. The EPA has stated that acute fish tests are inappropriate for compounds with log $K_{o/w}$ values above 4.2, and it recommends that with such highly hydrophobic compounds a chronic *Daphnia* test be used instead of the acute fish test (EPA, *Federal Register* 2000, pp. 81679, 81695). The log $K_{o/w}$ value of 2,5-fu has been calculated to be 5.36, and is to be measured experimentally as part of the tests (test plan, pp. 6-7). It is therefore definitely premature, and most likely inappropriate, to carry out a fish test. If the ACC wishes to

investigate the acute fish toxicity of 2,5-fu, we urge it to use one or more of the several available *in vitro* and *in silico* methods (see Appendix I).

Second, the *in vivo* micronucleus genotoxicity test is inappropriate, as it can readily be replaced with *in vitro* genetic toxicity tests, including the chromosomal aberration test (OECD 473). This test is most commonly carried out using Chinese hamster ovary cells, in which case it is not strictly a non-animal test. However, human lymphocytes can equally readily be used. In a 1999 letter to HPV program participants and again in its December 2000 *Federal Register* notice, the EPA made the following statement: "Participants are encouraged to use *in vitro* genetic toxicity testing to generate any needed genetic toxicity screening data, unless known chemical properties preclude its use" (Wayland 1999). The EPA's guidance has clearly not been followed in the case of 2,5-fu, as the ACC has made no attempt to explain how known chemical properties preclude the use of *in vitro* genetic toxicity tests in this case. Indeed, the ACC acknowledges that "The most frequently used test systems investigate changes in mammalian cells … following either *in vitro* or *in vivo* exposure to the test substance", but then, with no explanation as to why the *in vitro* approach cannot be used, the ACC simply states that "The micronucleus test is a common *in vivo* assay" (test plan, p. 13). The EPA must ask the ACC for justification for conducting an *in vivo* test for genetic toxicity.

Finally, with respect to the OECD 422, the ACC may well be correct in stating that no non-acute mammalian toxicity data are available. However, the absence of these toxicity data does not directly translate into the necessity to carry out additional animal experiments, for the reasons stated in our general comments above. Further, no attempt to categorize 2,5-fu with similar compounds appears to have been made. The information provided about the chemistry of 2,5-fu is both very limited and inconsistent. This lack of attention to the compound's chemistry strongly suggests that little attention has been given to the potential for categorization, which represents a deviation from the EPA's instruction that "Participants shall maximize the use of scientifically appropriate categories of related chemicals and structure activity relationships" (Wayland 1999). We therefore suggest that the ACC estimate the toxicity of 2,5-fu by means of detailed structural analysis. One obvious approach is to search for toxicity data for similar compounds.

We urge the ACC to apply thoughtful toxicology and spare another 875 animals the pain and suffering involved in these unnecessary experiments. Should you have any questions, I can be reached at 757-622-7382, ext. 1304 or by email at JessicaS@PETA.org.

Sincerely,

Jessica Sandler
Federal Agency Liaison

Appendix I: Alternatives to the acute fish toxicity test

With respect to *in silico* methods, several quantitative structure activity relationship (QSAR) programs for estimating toxicity to fish and other aquatic organisms are available. The EPA itself encourages the use of one established QSAR: ECOSAR (EPA 2002).

With respect to *in vitro* methods, TETRATOX, an assay based on the *protozoan Tetrahymena pyriformis* (Larsen 1997), is the most appropriate. With 50% growth impairment as the endpoint, the results of this assay show close similarity to toxicity in the fathead minnow (Schultz 1997). The extensive available information demonstrates that TETRATOX is an effective alternative to fish testing. It is in fact already used extensively in industry, and is being considered for regulatory acceptance by the OECD. It is also rapid, easy to use, and inexpensive. On October 23, 2001, PETA and PCRM held a meeting with EPA to facilitate incorporation of an *in vitro* aquatic toxicity test into the HPV program, and Dr. Schultz (Professor of Predictive Toxicology, University of Tennessee College of Veterinary Medicine) made a presentation about TETRATOX. On December 5, 2001, PCRM scientist Nicole Cardello presented the details of this meeting, and our proposal, in a letter to EPA Assistant Administrator Stephen Johnson. After almost two years, there has still been no response from Mr. Johnson or anyone else in the agency. We again request a thoughtful, scientific and specific reply to this letter. It is the stated goal of the EPA to incorporate *in vitro* methods into the HPV program, and this presents an ideal opportunity for action rather than words.

The recently validated *Dar*T test is another prospective replacement for *in vivo* studies. The test protocol and performance parameters are described in detail in Schulte (1994) and Nagel (1998). Briefly, however, the *Dar*T test uses fertilized zebrafish (*Danio rerio*) eggs as a surrogate for living fish. The exposure period is 48 hours, and assessed endpoints include coagulation, blastula development, gastrulation, termination of gastrulation, development of somites, movement, tail extension, eye development, circulation, heart rate, pigmentation and edema. Endpoints comparable to *in vivo* lethality include failure to complete gastrulation after 12 hours, absence of somites after 16 hours, absence of heartbeat after 48 hours, and coagulated eggs. The other endpoints provide further insight for a more detailed assessment of test substances. The reliability and relevance of the *Dar*T test have recently been confirmed in an international validation study coordinated and financed by the German Environmental Protection Agency, and predictions of acute toxicity from the *Dar*T test were highly concordant with *in vivo* reference data (Schulte 1996). This *in vitro* test has been accepted in Germany as a replacement for the use of fish in the assessment of wastewater effluent (Friccius 1995), and is clearly suitable for immediate use as a replacement for the use of fish in the HPV program's screening-level toxicity studies.

References

EPA, "Data collection and development on high production volume (HPV) chemicals", Federal Register, Vol. 65, No. 248, Dec. 26, 2000.

EPA, "Ecological structure activity relationships", Oct. 15, 2002, http://www.epa.gov/oppt/newchems/21ecosar.htm.

Friccius, T., *et al*., "Der Embryotest mit dem Zebrabärbling: Eine Neue Mögligkeit zur Prüfung und Bewertung der Toxizität von Abwasserproben", *Vom Wasse*r 84: 407-418, 1995.

Larsen, J., et al., "Progress in an ecotoxicological standard protocol with protozoa: Results from a pilot ring test with *Tetrahymena pyriformis*", *Chemosphere* 35: 1023-41, 1997.

Nagel, R., *Umweltchemikalien und Fische: Beiträge zu Einer Bewertung*, Johannes Gutenberg Universität, Mainz, 1998.

Schulte, C., *et al*., "Testing acute toxicity in the embryo in zebrafish, Brachydanio rerio, as an alternative to the acute fish test: Preliminary results", *Alternatives to Laboratory Animals* 22: 12-19, 1994.

Schulte, C., *et al*., "Testing acute toxicity in the embryo of zebrafish (*Brachydanio rerio*): An alternative to the acute fish toxicity test", *Proceedings of the 2nd World Congress on Alternatives and Animal Use in the Life Sciences*, Utrecht, Netherlands, 1996.

Schultz, T.W., "TETRATOX: *Tetrahymena pyriformis* population growth impairment endpoint - a surrogate for fish lethality", *Toxicology Methods* 7: 289-309, 1997.

Wayland, S.H., Letters to manufacturers/importers, Oct. 4, 1999, http://www.epa.gov/chemrtk/ceoltr2.htm.